

## WHAT IS CLAIMED:

1. A method of conducting a reduced dimensionality three-dimensional (3D) HA,CA,(CO),N,HN nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for the following nuclei of a protein molecule having two consecutive amino acid residues,  $i-1$  and  $i$ : (1) an  $\alpha$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}^\alpha_{i-1}$ ; (2) an  $\alpha$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}^\alpha_{i-1}$ ; (3) a polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  $^{15}\text{N}_i$ ; and (4) a polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}^\text{N}_i$ , wherein the chemical shift values of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  which are encoded in a peak pair of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  of amino acid residue  $i-1$  are connected to the chemical shift evolutions of  $^{15}\text{N}_i$  and  $^1\text{H}^\text{N}_i$  of amino acid residue  $i$ , under conditions effective (1) to generate NMR signals encoding the chemical shift values of  $^{13}\text{C}^\alpha_{i-1}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}\text{C}^\alpha)$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}^\text{N}_i$  in a direct time domain dimension,  $t_3(^1\text{H}^\text{N})$ , and (2) to sine modulate the  $^{13}\text{C}^\alpha_{i-1}$  chemical shift evolution in  $t_1(^{13}\text{C}^\alpha)$  with the chemical shift evolution of  $^1\text{H}^\alpha_{i-1}$ ; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with an anti-phase peak pair derived from said sine modulating, wherein (1) the chemical shift values of  $^{15}\text{N}_i$  and  $^1\text{H}^\text{N}_i$  are measured in two frequency domain dimensions,  $\omega_2(^{15}\text{N})$  and  $\omega_3(^1\text{H}^\text{N})$ , respectively, and (2) the chemical shift values of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^\alpha)$ , by the frequency difference between the two peaks forming said anti-phase peak pair and the frequency at the center of the two peaks, respectively, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift value of  $^1\text{H}^\alpha_{i-1}$  in a phase sensitive manner.

2. The method according to claim 1, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^{\alpha}_{i-1}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}^{\text{N}}_i$  in  $t_3(^1\text{H}^{\text{N}})$  and (2) to cosine modulate the  $^{13}\text{C}^{\alpha}_{i-1}$  chemical shift evolution in  $t_1(^{13}\text{C}^{\alpha})$  with the chemical shift evolution of  $^1\text{H}^{\alpha}_{i-1}$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with an in-phase peak pair derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

3. The method according to claim 1, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{15}\text{N}_i$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with a peak pair wherein (1) the chemical shift value of  $^1\text{H}^{\text{N}}_i$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^{\text{N}})$ , and (2) the chemical shift values of  $^1\text{H}^{\alpha}_{i-1}$  and  $^{13}\text{C}^{\alpha}_{i-1}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha})$ , by the frequency difference between the two peaks forming said peak pair and the frequency at the center of the two peaks, respectively.

4. The method according to claim 1, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ , occurs under conditions effective to generate NMR signals encoding the chemical shift value of  $^{13}\text{C}'_{i-1}$  in a phase sensitive manner in an indirect time domain dimension,  $t_4(^{13}\text{C}')$ , and said processing the NMR signals generates a four dimensional (4D)

NMR spectrum with a peak pair wherein (1) the chemical shift values of  $^{15}\text{N}_i$ ,  $^1\text{H}_i^{\text{N}}$  and  $^{13}\text{C}_{i-1}'$  are measured in three frequency domain dimensions,  $\omega_2(^{15}\text{N})$ ,  $\omega_3(^1\text{H}^{\text{N}})$ , and  $\omega_4(^{13}\text{C}')$ , respectively, and (2) the chemical shift values of  $^1\text{H}_{i-1}^{\alpha}$  and  $^{13}\text{C}_{i-1}^{\alpha}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha})$ , by the frequency difference between the two peaks forming said peak pair and the frequency at the center of the two peaks, respectively.

5. The method according to claim 1, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}_{i-1}^{\alpha}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}_i^{\text{N}}$  in  $t_3(^1\text{H}^{\text{N}})$ , and (2) to avoid sine modulating the  $^{13}\text{C}_{i-1}^{\alpha}$  chemical shift evolution in  $t_1(^{13}\text{C}^{\alpha})$  with the chemical shift evolution of  $^1\text{H}_{i-1}^{\alpha}$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals generates a 3D NMR spectrum with an additional peak located centrally between two peaks forming said peak pair which measures the chemical shift value of  $^{13}\text{C}_{i-1}^{\alpha}$  along  $\omega_1(^{13}\text{C}^{\alpha})$ .

6. The method according to claim 5, wherein said additional peak is derived from  $^{13}\text{C}^{\alpha}$  nuclear spin polarization.

7. The method according to claim 6, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2B, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}_{i-1}^{\alpha}$  magnetization, which is transferred to  $^{13}\text{C}_{i-1}^{\alpha}$ , to  $^{15}\text{N}_i$ , and to  $^1\text{H}_i^{\text{N}}$ , to generate the NMR signal.

8. The method according to claim 7, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3B to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3B, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pair and a second NMR subspectrum derived from said adding which contains said additional peak located centrally between the two peaks forming said peak pair.

9. A method of conducting a reduced dimensionality three-dimensional (3D)  $\underline{\text{H}}, \underline{\text{C}}, -(\text{C-TOCSY-CO}), \text{N}, \text{HN}$  nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for the following nuclei of a protein molecule having two consecutive amino acid residues,  $i-1$  and  $i$ : (1) aliphatic protons of amino acid residue  $i-1$ ,  $^1\text{H}_{i-1}^{\text{ali}}$ ; (2) aliphatic carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}_{i-1}^{\text{ali}}$ ; (3) a polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  $^{15}\text{N}_i$ ; and (4) a polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}_i^{\text{N}}$ , wherein the chemical shift values of  $^1\text{H}_{i-1}^{\text{ali}}$  and  $^{13}\text{C}_{i-1}^{\text{ali}}$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}_{i-1}^{\text{ali}}$  and  $^{13}\text{C}_{i-1}^{\text{ali}}$  of amino acid residue  $i-1$  are connected to the chemical shift evolutions of  $^{15}\text{N}_i$  and  $^1\text{H}_i^{\text{N}}$  of amino acid residue  $i$ , under conditions effective (1) to generate a NMR signal encoding the chemical shift values of  $^{13}\text{C}_{i-1}^{\text{ali}}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}\text{C}^{\text{ali}})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}_i^{\text{N}}$  in a direct time domain dimension,  $t_3(^1\text{H}^{\text{N}})$ , and (2) to sine modulate the chemical shift evolutions of  $^{13}\text{C}_{i-1}^{\text{ali}}$  in  $t_1(^{13}\text{C}^{\text{ali}})$  with the chemical shift evolutions of  $^1\text{H}_{i-1}^{\text{ali}}$ ; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein (1) the chemical shift values of  $^{15}\text{N}_i$  and  $^1\text{H}_i^{\text{N}}$  are measured in two frequency

domain dimensions,  $\omega_2(^{15}\text{N})$  and  $\omega_3(^1\text{H}^{\text{N}})$ , respectively, and (2) the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\text{ali}})$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs and the frequencies at the center of the two peaks, respectively, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift value of  $^1\text{H}^{\text{ali}}_{i-1}$  in a phase sensitive manner.

10. The method according to claim 9, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^{\text{ali}}_{i-1}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\text{ali}})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}^{\text{N}}_i$  in  $t_3(^1\text{H}^{\text{N}})$  and (2) to cosine modulate the chemical shift evolutions of  $^{13}\text{C}^{\text{ali}}_{i-1}$  in  $t_1(^{13}\text{C}^{\text{ali}})$  with the chemical shift evolutions of  $^1\text{H}^{\text{ali}}_{i-1}$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

11. The method according to claim 9, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{15}\text{N}_i$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with peak pairs wherein (1) the chemical shift value of  $^1\text{H}^{\text{N}}_i$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^{\text{N}})$ , and (2) the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\text{ali}})$ , by the frequency differences between the two peaks forming said peak pairs and the frequencies at the center of the two peaks, respectively.

12. The method according to claim 9, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ , occurs under conditions effective to generate NMR signals encoding the chemical shift value of  $^{13}\text{C}'_{i-1}$  in a phase sensitive manner in an indirect time domain dimension,  $t_4(^{13}\text{C}')$ , and said processing the NMR signals generates a four dimensional (4D) NMR spectrum with variant peak pairs wherein (1) the chemical shift values of  $^{15}\text{N}_i$ ,  $^1\text{H}^{\text{N}}_i$  and  $^{13}\text{C}'_{i-1}$  are measured in three frequency domain dimensions,  $\omega_2(^{15}\text{N})$ ,  $\omega_3(^1\text{H}^{\text{N}})$ , and  $\omega_4(^{13}\text{C}')$ , respectively, and (2) the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\text{ali}})$ , by the frequency differences between the two peaks forming said variant peak pairs and the frequencies at the center of the two peaks, respectively.

13. The method according to claim 9, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^{\text{ali}}_{i-1}$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\text{ali}})$  and  $t_2(^{15}\text{N})$  and the chemical shift value of  $^1\text{H}^{\text{N}}_i$  in  $t_3(^1\text{H}^{\text{N}})$ , and (2) to avoid sine modulating the chemical shift evolutions of  $^{13}\text{C}^{\text{ali}}_{i-1}$  in  $t_1(^{13}\text{C}^{\text{ali}})$  with the chemical shift evolution of  $^1\text{H}^{\alpha}_{i-1}$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals generates a 3D NMR spectrum with additional peaks located centrally between said peak pairs which measure the chemical shift values of  $^{13}\text{C}^{\text{ali}}_{i-1}$  along  $\omega_1(^{13}\text{C}^{\text{ali}})$ .

14. The method according to claim 13, wherein said additional peaks are derived from  $^{13}\text{C}^{\text{ali}}$  nuclear spin polarization.

15. The method according to claim 14, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2C, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^{\text{ali}}_{i-1}$  magnetization, and  $^1\text{H}^{\text{ali}}_{i-1}$  magnetization is transferred to  $^{13}\text{C}^{\text{ali}}_{i-1}$ , to  $^{13}\text{C}^{\alpha}_{i-1}$ , to  $^{13}\text{C}'_{i-1}$ , to  $^{15}\text{N}_i$ , and to  $^1\text{H}^{\text{N}}_i$ , where the NMR signal is detected.

16. The method according to claim 15, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3C to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3C, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pairs, and a second NMR subspectrum derived from said adding which contains said additional peaks located centrally between said peak pairs.

17. A method of conducting a reduced dimensionality three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for the following nuclei of a protein molecule having an amino acid residue,  $i$ : (1) a  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ ; (2) a  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ ; (3) an  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ ; (4) an  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ ; and (5) a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$ , wherein the chemical shift values of  $^1\text{H}^\alpha_i/^{13}\text{C}^\alpha_i$  and  $^1\text{H}^\beta_i/^{13}\text{C}^\beta_i$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}^\alpha_i$ ,  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\alpha_i$ , and  $^{13}\text{C}^\beta_i$  are connected to the chemical shift evolution of  $^{13}\text{C}'_i$ , under conditions effective (1) to generate NMR signals encoding the chemical shift values of  $^{13}\text{C}^\alpha_i$ ,  $^{13}\text{C}^\beta_i$  and  $^{13}\text{C}'_i$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{13}\text{C}')$ , respectively, and the chemical shift value of  $^1\text{H}^\alpha_i$  in a direct time domain dimension,  $t_3(^1\text{H}^\alpha)$  and (2) to sine modulate

the chemical shift evolutions of  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$ , respectively; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein (1) the chemical shift values of  $^{13}\text{C}'_i$  and  $^1\text{H}^{\alpha}_i$  are measured in two frequency domain dimensions,  $\omega_2(^{13}\text{C}')$  and  $\omega_3(^1\text{H}^{\alpha})$ , respectively, and (2) (i) the chemical shift values of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs, and (ii) the chemical shift values of  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequencies at the center of the two peaks forming said anti-phase peak pairs, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift values of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  in a phase sensitive manner.

18. The method according to claim 17, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^{\alpha}_i$ ,  $^{13}\text{C}^{\beta}_i$  and  $^{13}\text{C}'_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{13}\text{C}')$ , respectively, and the chemical shift value of  $^1\text{H}^{\alpha}_i$  in  $t_3(^1\text{H}^{\alpha})$  and (2) to cosine modulate the  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  chemical shift evolutions in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  for the additional NMR signals and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.



19. The method according to claim 17, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{13}\text{C}'_i$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with peak pairs wherein (1) the chemical shift value of  $^1\text{H}^\alpha_i$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^\alpha)$ , and (2) (i) the chemical shift values of  $^1\text{H}^\alpha_i$  and  $^1\text{H}^\beta_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequency differences between two peaks forming said peak pairs, respectively, and (ii) the chemical shift values of  $^{13}\text{C}^\alpha_i$  and  $^{13}\text{C}^\beta_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequencies at the center of the two peaks forming said peak pairs.

20. The method according to claim 17 wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^\alpha_i$ ,  $^{13}\text{C}^\beta_i$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{15}\text{N})$  and the chemical shift value of  $^1\text{H}^\alpha_i$  in  $t_3(^1\text{H}^\alpha)$ , and (2) to avoid sine modulating the chemical shift evolutions of  $^{13}\text{C}^\alpha_i$  and  $^{13}\text{C}^\beta_i$  in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}^\alpha_i$  and  $^1\text{H}^\beta_i$  for the additional NMR signal, and said processing the NMR signals and the additional NMR signals generates a 3D NMR spectrum with additional peaks located centrally between the two peaks forming said peak pairs which measure the chemical shift values of  $^{13}\text{C}^\alpha_i$  and  $^{13}\text{C}^\beta_i$  along  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ .

21. The method according to claim 20, wherein said additional peaks are derived from  $^{13}\text{C}^\alpha$  and  $^{13}\text{C}^\beta$  nuclear spin polarization.

22. The method according to claim 21, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2E, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^\alpha_i$  and  $^1\text{H}^\beta_i$  magnetization, and  $^1\text{H}^\alpha_i$  and  $^1\text{H}^\beta_i$  polarization is transferred to  $^{13}\text{C}^\alpha_i$  and  $^{13}\text{C}^\beta_i$ , to  $^{13}\text{C}'_i$ , and back to  $^1\text{H}^\alpha_i$ , where the NMR signal is detected.

23. The method according to claim 22, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3E to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3E, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pairs, and a second NMR subspectrum derived from said adding which contains said additional peaks located centrally between the two peaks forming said peak pairs.

24. A method of conducting a reduced dimensionality three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for the following nuclei of a protein molecule having an amino acid residue,  $i$ : (1) a  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}_i^\beta$ ; (2) a  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}_i^\beta$ ; (3) an  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}_i^\alpha$ ; (4) an  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}_i^\alpha$ ; (5) a polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  $^{15}\text{N}_i$ ; and (6) a polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}_i^{\text{N}}$ , wherein the chemical shift values of  $^1\text{H}_i^\alpha/^{13}\text{C}_i^\alpha$  and  $^1\text{H}_i^\beta/^{13}\text{C}_i^\beta$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}_i^\alpha$ ,  $^1\text{H}_i^\beta$ ,  $^{13}\text{C}_i^\alpha$ , and  $^{13}\text{C}_i^\beta$  are connected to the chemical shift evolutions of  $^{15}\text{N}_i$  and  $^1\text{H}_i^{\text{N}}$ , under conditions effective (1) to generate NMR signals encoding the chemical shift values of  $^{13}\text{C}_i^\alpha$ ,  $^{13}\text{C}_i^\beta$  and  $^{15}\text{N}_i$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}_i^{\text{N}}$  in a direct time domain dimension,  $t_3(^1\text{H}^{\text{N}})$  and (2) to

sine modulate the chemical shift evolutions of  $^{13}\text{C}_i^\alpha$  and  $^{13}\text{C}_i^\beta$  in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}_i^\alpha$  and  $^1\text{H}_i^\beta$ , respectively; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein (1) the chemical shift values of  $^{15}\text{N}_i$  and  $^1\text{H}_i^\text{N}$  are measured in two frequency domain dimensions,  $\omega_2(^{15}\text{N})$  and  $\omega_3(^1\text{H}^\text{N})$ , respectively, and (2) (i) the chemical shift values of  $^1\text{H}_i^\alpha$  and  $^1\text{H}_i^\beta$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs, and (ii) the chemical shift values of  $^{13}\text{C}_i^\alpha$  and  $^{13}\text{C}_i^\beta$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequencies at the center of said two peaks forming said anti-phase peak pairs, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift values of  $^1\text{H}_i^\alpha$  and  $^1\text{H}_i^\beta$  in a phase sensitive manner.

25. The method according to claim 24, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}_i^\alpha$ ,  $^{13}\text{C}_i^\beta$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{15}\text{N})$ , respectively, and the chemical shift value of  $^1\text{H}_i^\text{N}$  in  $t_3(^1\text{H}^\text{N})$  and (2) to cosine modulate the  $^{13}\text{C}_i^\alpha$  and  $^{13}\text{C}_i^\beta$  chemical shift evolutions in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}_i^\alpha$  and  $^1\text{H}_i^\beta$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

26. The method according to claim 24, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{15}\text{N}_i$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with peak pairs wherein (1) the chemical shift value of  $^1\text{H}^{\text{N}}_i$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^{\text{N}})$ , and (2) (i) the chemical shift values of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequency differences between the two peaks forming said peak pairs, and (ii) the chemical shift values of  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ , by the frequencies at the center of the two peaks forming said peak pairs.

27. The method according to claim 24, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^{\alpha}_i$ ,  $^{13}\text{C}^{\beta}_i$  and  $^{15}\text{N}_i$  in a phase sensitive manner in  $t_1(^{13}\text{C}^{\alpha/\beta})$  and  $t_2(^{15}\text{N})$  and the chemical shift value of  $^1\text{H}^{\text{N}}_i$  in  $t_3(^1\text{H}^{\text{N}})$ , and (2) to avoid sine modulating the chemical shift evolutions of  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  in  $t_1(^{13}\text{C}^{\alpha/\beta})$  with the chemical shift evolutions of  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals generates a 3D NMR spectrum with additional peaks located centrally between the two peaks forming said peak pairs which measure the chemical shift values of  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$  along  $\omega_1(^{13}\text{C}^{\alpha/\beta})$ .

28. The method according to claim 27, wherein said additional peaks are derived from  $^{13}\text{C}^{\alpha}$  and  $^{13}\text{C}^{\beta}$  nuclear spin polarization.

29. The method according to claim 28, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2F, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  magnetization, and  $^1\text{H}^{\alpha}_i$  and  $^1\text{H}^{\beta}_i$  magnetization is transferred to  $^{13}\text{C}^{\alpha}_i$  and  $^{13}\text{C}^{\beta}_i$ , to  $^{15}\text{N}_i$ , and to  $^1\text{H}^{\text{N}}_i$ , where the NMR signal is detected.

30. The method according to claim 29, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3F to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3F, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pairs, and a second NMR subspectrum derived from said adding which contains said additional peaks located centrally between the two peaks forming said peak pairs.

31. A method of conducting a reduced dimensionality three-dimensional (3D)  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H}$ -COSY nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for  $^1\text{H}^m$ ,  $^{13}\text{C}^m$ ,  $^1\text{H}^n$ , and  $^{13}\text{C}^n$  of a protein molecule wherein  $m$  and  $n$  indicate atom numbers of two CH,  $\text{CH}_2$  or  $\text{CH}_3$  groups that are linked by a single covalent carbon-carbon bond in an amino acid residue, wherein the chemical shift values of  $^1\text{H}^m$  and  $^{13}\text{C}^m$  which are encoded in a peak pair of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}^m$  and  $^{13}\text{C}^m$  are connected to the chemical shift evolutions of  $^1\text{H}^n$  and  $^{13}\text{C}^n$ , under conditions effective (1) to generate NMR signals encoding the chemical shift values of  $^{13}\text{C}^m$  and  $^{13}\text{C}^n$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}\text{C}^m)$  and  $t_2(^{13}\text{C}^n)$ , respectively, and the chemical shift value of  $^1\text{H}^n$  in a direct time domain dimension,  $t_3(^1\text{H}^n)$ , and (2) to sine modulate the chemical shift evolution of  $^{13}\text{C}^m$  in  $t_1(^{13}\text{C}^m)$  with the chemical shift evolution of  $^1\text{H}_m$ ; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein

(1) the chemical shift values of  $^{13}\text{C}^n$  and  $^1\text{H}^n$  are measured in two frequency domain dimensions,  $\omega_2(^{13}\text{C}^n)$  and  $\omega_3(^1\text{H}^n)$ , respectively, and (2) the chemical shift values of  $^1\text{H}^m$  and  $^{13}\text{C}^m$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^m)$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs and the frequencies at the center of the two peaks, respectively, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift value of  $^1\text{H}_m$  in a phase sensitive manner.

32. The method according to claim 31, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^m$  and  $^{13}\text{C}^n$  in a phase sensitive manner in  $t_1(^{13}\text{C}^m)$  and  $t_2(^{13}\text{C}^n)$ , respectively, and the chemical shift value of  $^1\text{H}^n$  in  $t_3(^1\text{H}^n)$ , and (2) to cosine modulate the  $^{13}\text{C}^m$  chemical shift evolution in  $t_1(^{13}\text{C}^m)$  with the chemical shift evolution of  $^1\text{H}_m$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

33. The method according to claim 31, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{13}\text{C}^n$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with peak pairs wherein (1) the chemical shift value of  $^1\text{H}^n$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^n)$ , and (2) the chemical shift values of  $^1\text{H}^m$  and  $^{13}\text{C}^m$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^m)$ ,

by the frequency differences between the two peaks forming said peak pairs and the frequencies at the center of the two peaks, respectively.

34. The method according to claim 31, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate an additional NMR signal encoding the chemical shift values of  $^{13}\text{C}^m$  and  $^{13}\text{C}^n$  in a phase sensitive manner in  $t_1(^{13}\text{C}^m)$  and  $t_2(^{13}\text{C}^n)$  and the chemical shift value of  $^1\text{H}^n$  in  $t_3(^1\text{H})$ , and (2) to avoid sine modulating the chemical shift evolution of  $^{13}\text{C}^m$  in  $t_1(^{13}\text{C}^m)$  with the chemical shift evolution of  $^1\text{H}^m$  for the additional NMR signal, and said processing the NMR signals and the additional NMR signal generates a 3D NMR spectrum with additional peaks located centrally between the two peaks forming said peak pairs which measure the chemical shift value of  $^{13}\text{C}^m$  along  $\omega_1(^{13}\text{C}^m)$ .

35. The method according to claim 34, wherein said additional peaks are derived from  $^{13}\text{C}^m$  nuclear spin polarization.

36. The method according to claim 35, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2H, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^m$  magnetization, and  $^1\text{H}^m$  magnetization is transferred to  $^{13}\text{C}^m$ , to  $^{13}\text{C}^n$ , and to  $^1\text{H}^n$ , where the NMR signal is detected.

37. The method according to claim 36, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3H to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3H, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains

said peak pairs, and a second NMR subspectrum derived from said adding which contains said additional peaks located centrally between the two peaks forming said peak pairs.

38. A method of conducting a reduced dimensionality three-dimensional (3D)  $\underline{H}, \underline{C}, \underline{C}, \underline{H}$ -TOCSY nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for  $^1H^m$ ,  $^{13}C^m$ ,  $^1H^n$ , and  $^{13}C^n$  of a protein molecule wherein  $m$  and  $n$  indicate atom numbers of two CH, CH<sub>2</sub> or CH<sub>3</sub> groups that may or may not be directly linked by a single covalent carbon-carbon bond in an amino acid residue, wherein the chemical shift values of  $^1H^m$  and  $^{13}C^m$  which are encoded in a peak pair of a 3D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1H^m$  and  $^{13}C^m$  are connected to the chemical shift evolutions of  $^1H^n$  and  $^{13}C^n$ , under conditions effective (1) to generate NMR signals encoding the chemical shift values of  $^{13}C^m$  and  $^{13}C^n$  in a phase sensitive manner in two indirect time domain dimensions,  $t_1(^{13}C^m)$  and  $t_2(^{13}C^n)$ , respectively, and the chemical shift value of  $^1H^n$  in a direct time domain dimension,  $t_3(^1H^n)$ , and (2) to sine modulate the chemical shift evolution of  $^{13}C^m$  in  $t_1(^{13}C^m)$  with the chemical shift evolution of  $^1H^m$ ; and

processing the NMR signals to generate a sine-modulated 3D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein (1) the chemical shift values of  $^{13}C^n$  and  $^1H^n$  are measured in two frequency domain dimensions,  $\omega_2(^{13}C^n)$  and  $\omega_3(^1H^n)$ , respectively, and (2) the chemical shift values of  $^1H^m$  and  $^{13}C^m$  are measured in a frequency domain dimension,  $\omega_1(^{13}C^m)$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs and the frequencies at the center of the two peaks, respectively, wherein said sine-modulated 3D NMR spectrum enables detection of the chemical shift value of  $^1H_m$  and in a phase sensitive manner.

39. The method according to claim 38, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate



additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^m$  and  $^{13}\text{C}^n$  in a phase sensitive manner in  $t_1(^{13}\text{C}^m)$  and  $t_2(^{13}\text{C}^n)$ , respectively, and the chemical shift value of  $^1\text{H}^n$  in  $t_3(^1\text{H}^n)$  and (2) to cosine modulate the  $^{13}\text{C}^m$  chemical shift evolution in  $t_1(^{13}\text{C}^m)$  with the chemical shift evolution of  $^1\text{H}^m$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 3D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 3D NMR spectrum generated by adding said sine-modulated 3D NMR spectrum and said cosine-modulated 3D NMR spectrum, and a difference 3D NMR spectrum generated by subtracting said cosine-modulated 3D NMR spectrum from said sine-modulated 3D NMR spectrum, wherein combined use of said sum 3D NMR spectrum and said difference 3D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

40. The method according to claim 38, wherein said applying radiofrequency pulses is carried out so that the chemical shift evolution of  $^{13}\text{C}^n$  does not occur and said processing the NMR signals generates a two dimensional (2D) NMR spectrum with peak pairs wherein (1) the chemical shift value of  $^1\text{H}^n$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^n)$ , and (2) the chemical shift values of  $^1\text{H}^m$  and  $^{13}\text{C}^m$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^m)$ , by the frequency differences between the two peaks forming said peak pairs and the frequencies at the center of the two peaks, respectively.

41. The method according to claim 38, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift values of  $^{13}\text{C}^m$  and  $^{13}\text{C}^n$  in a phase sensitive manner in  $t_1(^{13}\text{C}^m)$  and  $t_2(^{13}\text{C}^n)$  and the chemical shift value of  $^1\text{H}^n$  in  $t_3(^1\text{H}^n)$ , and (2) to avoid sine modulating the chemical shift evolution of  $^{13}\text{C}^m$  in  $t_1(^{13}\text{C}^m)$  with the chemical shift evolution of  $^1\text{H}^m$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals generates a 3D NMR spectrum with additional peaks located centrally between the two peaks

forming said peak pairs which measure the chemical shift value of  $^{13}\text{C}^m$  along  $\omega_1(^{13}\text{C}^m)$ .

42. The method according to claim 41, wherein said additional peaks are derived from  $^{13}\text{C}^m$  nuclear spin polarization.

43. The method according to claim 42, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2I, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^m$  magnetization, and  $^1\text{H}^m$  magnetization is transferred to  $^{13}\text{C}^m$ , to  $^{13}\text{C}^n$ , and to  $^1\text{H}^n$ , where the NMR signal is detected.

44. The method according to claim 43, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3I to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3I, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pairs, and a second NMR subspectrum derived from said adding which contains said additional peaks located centrally between the two peaks forming said peak pairs.

45. A method of conducting a reduced dimensionality two-dimensional (2D) HB, CB, (CG, CD), HD nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for the following nuclei of a protein molecule : (1) a  $\beta$ -proton of an amino acid residue with an aromatic side chain,  $^1\text{H}^\beta$ ; (2) a  $\beta$ -carbon of an amino acid residue with an aromatic side chain,  $^{13}\text{C}^\beta$ ; and (3) a  $\delta$ -proton of an amino acid residue with an aromatic side chain,  $^1\text{H}^\delta$ ,

wherein the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  which are encoded in a peak pair of a 2D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  are connected to the chemical shift evolution of  $^1\text{H}^\delta$ , under conditions effective (1) to generate NMR signals encoding the chemical shift value of  $^{13}\text{C}^\beta$  in a phase sensitive manner in an indirect time domain dimension,  $t_1(^{13}\text{C}^\beta)$ , and the chemical shift value of  $^1\text{H}^\delta$  in a direct time domain dimension,  $t_2(^1\text{H}^\delta)$ , and (2) to sine modulate the chemical shift evolution of  $^{13}\text{C}^\beta$  in  $t_1(^{13}\text{C}^\beta)$  with the chemical shift evolution of  $^1\text{H}^\beta$ ; and

processing the NMR signals to generate a sine-modulated 2D NMR spectrum with an anti-phase peak pair derived from said sine modulating wherein (1) the chemical shift value of  $^1\text{H}^\delta$  is measured in a frequency domain dimension,  $\omega_2(^1\text{H}^\delta)$ , and (2) the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^\beta)$ , by the frequency difference between the two peaks forming said anti-phase peak pair and the frequency at the center of the two peaks, respectively, wherein said sine-modulated 2D NMR spectrum enables detection of the chemical shift value of  $^1\text{H}^\beta$  in a phase sensitive manner.

46. The method according to claim 45, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift value of  $^{13}\text{C}^\beta$  in a phase sensitive manner in  $t_1(^{13}\text{C}^\beta)$  and the chemical shift value of  $^1\text{H}^\delta$  in  $t_2(^1\text{H}^\delta)$  and (2) to cosine modulate the  $^{13}\text{C}^\beta$  chemical shift evolution in  $t_1(^{13}\text{C}^\beta)$  with the chemical shift evolution of  $^1\text{H}^\beta$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals further comprises generating a cosine-modulated 2D NMR spectrum with an in-phase peak pair derived from said cosine modulating, a sum 2D NMR spectrum generated by adding said sine-modulated 2D NMR spectrum and said cosine-modulated 2D NMR spectrum, and a difference 2D NMR spectrum generated by subtracting said cosine-modulated

2D NMR spectrum from said sine-modulated 2D NMR spectrum, wherein combined use of said sum 2D NMR spectrum and said difference 2D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

47. The method according to claim 45, wherein said applying radiofrequency pulses is carried out so that:

(i) the chemical shift evolution of a  $\delta$ -carbon of an amino acid residue with an aromatic side chain,  $^{13}\text{C}^\delta$ , occurs under conditions effective to generate NMR signals encoding the chemical shift value of  $^{13}\text{C}^\delta$  in a phase sensitive manner in an indirect time domain dimension,  $t_3(^{13}\text{C}^\delta)$ , and said processing the NMR signals generates a three dimensional (3D) NMR spectrum with a peak pair wherein (1) the chemical shift values of  $^1\text{H}^\delta$  and  $^{13}\text{C}^\delta$  are measured in two frequency domain dimensions,  $\omega_2(^1\text{H}^\delta)$  and  $\omega_3(^{13}\text{C}^\delta)$ , respectively, and (2) the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^\beta)$ , by the frequency difference between the two peaks forming said peak pair and the frequency at the center of the two peaks, respectively; or

(ii) the chemical shift evolution of a  $\gamma$ -carbon of an amino acid residue with an aromatic side chain,  $^{13}\text{C}^\gamma$  occurs under conditions effective to generate NMR signals encoding the chemical shift value of  $^{13}\text{C}^\gamma$ , in a phase sensitive manner in an indirect time domain dimension,  $t_3(^{13}\text{C}^\gamma)$ , and said processing the NMR signals generates a three dimensional (3D) NMR spectrum with a peak pair wherein (1) the chemical shift values of  $^1\text{H}^\delta$  and  $^{13}\text{C}^\gamma$  are measured in two frequency domain dimensions,  $\omega_2(^1\text{H}^\delta)$  and  $\omega_3(^{13}\text{C}^\gamma)$ , respectively, and (2) the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  are measured in a frequency domain dimension,  $\omega_1(^{13}\text{C}^\beta)$ , by the frequency difference between the two peaks forming said peak pair and the frequency at the center of the two peaks, respectively.

48. The method according to claim 46, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift value of  $^{13}\text{C}^\beta$  in a phase sensitive manner in  $t_1(^{13}\text{C}^\beta)$  and the chemical shift value of  $^1\text{H}^\delta$  in  $t_2(^1\text{H}^\delta)$ , and (2) to avoid sine modulating the chemical shift evolution of  $^{13}\text{C}^\beta$  in  $t_1(^{13}\text{C}^\beta)$  with the chemical shift evolution of  $^1\text{H}^\beta$  for the additional NMR signals, and said processing the NMR signals and the additional NMR signals generates a 2D NMR spectrum with an additional peak located centrally between said peak pair which measure the chemical shift value of  $^{13}\text{C}^\beta$  along  $\omega_1(^{13}\text{C}^\beta)$ .

49. The method according to claim 48, wherein said additional peak is derived from  $^{13}\text{C}^\beta$  nuclear spin polarization.

50. The method according to claim 49, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2J, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^\beta$  magnetization, and  $^1\text{H}^\beta$  magnetization is transferred to  $^{13}\text{C}^\beta$ , to  $^{13}\text{C}^\delta$ , and to  $^1\text{H}^\delta$ , where the NMR signal is detected.

51. The method according to claim 50, wherein said applying radiofrequency pulses comprises:

applying a first set of radiofrequency pulses according to the scheme shown in Figure 3J to generate a first NMR signal, and

applying a second set of radiofrequency pulses according to the scheme shown in Figure 3J, wherein phase  $\phi_1$  of the first  $^1\text{H}$  pulse is altered by  $180^\circ$  to generate a second NMR signal, said method further comprising:

adding and subtracting the first NMR signal and the second NMR signal prior to said processing, whereby said processing the NMR signals generates a first NMR subspectrum derived from said subtracting which contains said peak pair, and a second NMR subspectrum derived from said adding which contains said additional peak located centrally between the two peaks forming said peak pair.

52. A method of conducting a reduced dimensionality two-dimensional (2D)  ${}^1\text{H}$ - ${}^{13}\text{C}$ -H-COSY nuclear magnetic resonance (NMR) experiment by measuring the chemical shift values for  ${}^1\text{H}^m$ ,  ${}^{13}\text{C}^m$ , and  ${}^1\text{H}^n$  of a protein molecule wherein  $m$  and  $n$  indicate atom numbers of two CH,  $\text{CH}_2$  or  $\text{CH}_3$  groups in an amino acid residue, wherein the chemical shift values of  ${}^1\text{H}^m$  and  ${}^{13}\text{C}^m$  which are encoded in a peak pair of a 2D NMR spectrum are detected in a phase sensitive manner, said method comprising:

providing a protein sample;

applying radiofrequency pulses to the protein sample which effect a nuclear spin polarization transfer wherein the chemical shift evolutions of  ${}^1\text{H}^m$  and  ${}^{13}\text{C}^m$  are connected to the chemical shift evolution of  ${}^1\text{H}^n$ , under conditions effective (1) to generate NMR signals encoding the chemical shift value of  ${}^{13}\text{C}^m$  in a phase sensitive manner in an indirect time domain dimension,  $t_1({}^{13}\text{C}^m)$ , and the chemical shift value of  ${}^1\text{H}^n$  in a direct time domain dimension,  $t_2({}^1\text{H}^n)$  and (2) to sine modulate the chemical shift evolution of  ${}^{13}\text{C}^m$  in  $t_1({}^{13}\text{C}^m)$  with the chemical shift evolution of  ${}^1\text{H}^m$ ; and

processing the NMR signals to generate a sine-modulated 2D NMR spectrum with anti-phase peak pairs derived from said sine modulating wherein (1) the chemical shift value of  ${}^1\text{H}^n$  is measured in a frequency domain dimension,  $\omega_2({}^1\text{H}^n)$ , and (2) the chemical shift values of  ${}^1\text{H}^m$  and  ${}^{13}\text{C}^m$  are measured in a frequency domain dimension,  $\omega_1({}^{13}\text{C}^m)$ , by the frequency differences between each of the two peaks forming each of said anti-phase peak pairs and the frequencies at the center of the two peaks, respectively, wherein said sine-modulated 2D NMR spectrum enables detection of the chemical shift value of  ${}^1\text{H}^m$  in a phase sensitive manner.

53. The method according to claim 52, wherein said applying radiofrequency pulses is carried out under conditions effective (1) to generate additional NMR signals encoding the chemical shift value of  ${}^{13}\text{C}^m$  in a phase sensitive manner in  $t_1({}^{13}\text{C}^m)$  and the chemical shift value of  ${}^1\text{H}^n$  in  $t_2({}^1\text{H}^n)$  and (2) to cosine modulate the  ${}^{13}\text{C}^m$  chemical shift evolution in  $t_1({}^{13}\text{C}^m)$  with the chemical shift evolution of  ${}^1\text{H}^m$  for the additional NMR signals, and said processing the

NMR signals and the additional NMR signals further comprises generating a cosine-modulated 2D NMR spectrum with in-phase peak pairs derived from said cosine modulating, a sum 2D NMR spectrum generated by adding said sine-modulated 2D NMR spectrum and said cosine-modulated 2D NMR spectrum, and a difference 2D NMR spectrum generated by subtracting said cosine-modulated 2D NMR spectrum from said sine-modulated 2D NMR spectrum, wherein combined use of said sum 2D NMR spectrum and said difference 2D NMR spectrum enables placement of the two peaks forming said peak pairs into separate spectra, thereby allowing phase-sensitive editing of the two peaks forming said peak pairs.

54. The method according to claim 52, wherein said applying radiofrequency pulses effects a nuclear spin polarization transfer according to Figure 2K, wherein a radiofrequency pulse is used to create transverse  $^1\text{H}^m$  magnetization, and  $^1\text{H}^m$  polarization is transferred to  $^{13}\text{C}^m$ , to  $^1\text{H}^m$ , and to  $^1\text{H}^n$ , where the NMR signal is detected.

55. The method according to claim 54, wherein said applying radiofrequency pulses is carried out according to the scheme shown in Figure 3K.

56. A method for sequentially assigning chemical shift values of an  $\alpha$ -proton,  $^1\text{H}^\alpha$ , an  $\alpha$ -carbon,  $^{13}\text{C}^\alpha$ , a polypeptide backbone amide nitrogen,  $^{15}\text{N}$ , and a polypeptide backbone amide proton,  $^1\text{H}^N$ , of a protein molecule comprising:

providing a protein sample;

conducting a set of reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of  $^1\text{H}^\alpha$  and  $^{13}\text{C}^\alpha$  which are encoded in a peak pair of a 3D NMR spectrum are detected in a phase sensitive manner, comprising: (1) a RD three dimensional (3D) HA,CA,(CO),N,HN NMR experiment to measure and connect chemical shift values of the  $\alpha$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}_{i-1}^\alpha$ , the  $\alpha$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}_{i-1}^\alpha$ , the polypeptide backbone amide nitrogen of amino acid

residue  $i$ ,  $^{15}\text{N}_i$ , and the polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}^{\text{N}}_i$  and (2) a RD 3D HNNCAHA NMR experiment to measure and connect the chemical shift values of the  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^\alpha$ ,  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  by (i) matching the chemical shift values of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  with the chemical shift values of  $^1\text{H}^\alpha_i$  and  $^{13}\text{C}^\alpha_i$ , (ii) using the chemical shift values of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  to identify the type of amino acid residue  $i-1$ , and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and using said chemical shift values to locate secondary structure elements within the polypeptide chain.

57. The method according to claim 56 further comprising:

subjecting the protein sample to a RD 3D H <sup>$\alpha/\beta$</sup> C <sup>$\alpha/\beta$</sup> (CO)NHN NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}^\beta_{i-1}$ , the  $\beta$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}^\beta_{i-1}$ ,  $^1\text{H}^\alpha_{i-1}$ ,  $^{13}\text{C}^\alpha_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  by using the chemical shift values of  $^1\text{H}^\beta_{i-1}$  and  $^{13}\text{C}^\beta_{i-1}$  to identify the type of amino acid residue  $i-1$ .

58. The method according to claim 57 further comprising:

subjecting the protein sample to a RD 3D H <sup>$\alpha/\beta$</sup> ,C <sup>$\alpha/\beta$</sup> ,CO,HA NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ , the  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_i$  by matching the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ , and  $^{13}\text{C}^\alpha_i$  measured by said RD 3D H <sup>$\alpha/\beta$</sup> ,C <sup>$\alpha/\beta$</sup> ,CO,HA NMR experiment with the sequentially assigned chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ ,  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  measured by said RD 3D HA,CA,(CO),N,HN NMR experiment, RD 3D HNNCAHA NMR experiment, and RD 3D H <sup>$\alpha/\beta$</sup> ,C <sup>$\alpha/\beta$</sup> (CO)NHN NMR experiment.



59. The method according to claim 57 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, N, HN$  NMR experiment to measure and connect the chemical shift values of  $^1H^{\beta}_i$ ,  $^{13}C^{\beta}_i$ ,  $^1H^{\alpha}_i$ ,  $^{13}C^{\alpha}_i$ ,  $^{15}N_i$ , and  $^1H^N_i$ ; and  
obtaining sequential assignments by matching the chemical shift values of  $^1H^{\beta}_i$ ,  $^{13}C^{\beta}_i$ ,  $^1H^{\alpha}_i$ , and  $^{13}C^{\alpha}_i$  with the chemical shift values of  $^1H^{\beta}_{i-1}$ ,  $^{13}C^{\beta}_{i-1}$ ,  $^1H^{\alpha}_{i-1}$ , and  $^{13}C^{\alpha}_{i-1}$  measured by said RD 3D  $\underline{H}^{\alpha/\beta} \underline{C}^{\alpha/\beta} (CO)NHN$  NMR experiment.
60. The method according to claim 57 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to measure and connect the chemical shift value of  $^{13}C^{\beta}_i$ ,  $^{13}C^{\alpha}_i$ ,  $^{15}N_i$ , and  $^1H^N_i$ ; and  
obtaining sequential assignments by matching the chemical shift values of  $^{13}C^{\beta}_i$  and  $^{13}C^{\alpha}_i$  measure by said 3D HNNCACB NMR experiment with the chemical shift values of  $^{13}C^{\beta}_{i-1}$  and  $^{13}C^{\alpha}_{i-1}$  measured by said RD 3D  $\underline{H}^{\alpha/\beta} \underline{C}^{\alpha/\beta} (CO)NHN$  NMR experiment.
61. The method according to claim 57 further comprising:  
subjecting the protein sample to a RD two-dimensional (2D)  $\underline{HB}, \underline{CB}, (CG, CD), HD$  NMR experiment to measure and connect the chemical shift values of  $^1H^{\beta}_{i-1}$ ,  $^{13}C^{\beta}_{i-1}$ , and a  $\delta$ -proton of amino acid residue  $i-1$  with an aromatic side chain,  $^1H^{\delta}_{i-1}$ ; and  
obtaining sequential assignments by (i) matching the chemical shift values of  $^1H^{\beta}_{i-1}$  and  $^{13}C^{\beta}_{i-1}$  measured by said RD 2D  $\underline{HB}, \underline{CB}, (CG, CD), HD$  NMR experiment with the chemical shift values of  $^1H^{\beta}$  and  $^{13}C^{\beta}$  measured by said RD 3D  $\underline{H}^{\alpha/\beta} \underline{C}^{\alpha/\beta} (CO)NHN$  NMR experiment, (ii) using said chemical shift values to identify amino acid residue  $i$  as having an aromatic side chain, and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and locating amino acid residues with aromatic side chains along said polypeptide chain.

62. The method according to claim 57 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}, \underline{C}, \underline{C}, \underline{H}$ -COSY NMR experiment or a RD 3D  $\underline{H}, \underline{C}, \underline{C}, \underline{H}$ -TOCSY NMR experiment to measure and connect the chemical shift values of aliphatic protons of amino acid residue  $i$ ,  $^1\text{H}^{\text{ali}}_i$ , and aliphatic carbons of amino acid residue  $i$ ,  $^{13}\text{C}^{\text{ali}}_i$ ; and  
obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$ , by (i) matching the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ , and  $^{13}\text{C}^\alpha_i$  measured using said RD 3D  $\underline{H}, \underline{C}, \underline{C}, \underline{H}$ -COSY NMR experiment or RD 3D  $\underline{H}, \underline{C}, \underline{C}, \underline{H}$ -TOCSY RD NMR experiment with the chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ , and  $^{13}\text{C}^\alpha$  measured by said RD 3D  $\underline{H}, \underline{A}, \underline{C}, \underline{A}, (\text{CO}), \text{N}, \text{HN}$  NMR experiment, RD 3D  $\text{HNNCAHA}$  NMR experiment, and RD 3D  $\underline{H}^{\alpha/\beta} \underline{C}^{\alpha/\beta} (\text{CO}) \text{NHN}$  NMR experiment and (ii) using the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$ , to identify the type of amino acid residue  $i$ .

63. The method according to claim 56 further comprising:  
subjecting the protein sample to a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the chemical shift values of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_{i-1}$  by matching the chemical shift value of  $^{13}\text{C}^\alpha_i$  measured by said RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment with the sequentially assigned chemical shift values of  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  measured by said RD 3D  $\underline{H}, \underline{A}, \underline{C}, \underline{A}, (\text{CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\text{HNNCAHA}$  NMR experiment.

64. The method according to claim 56 further comprising:  
subjecting the protein sample to (i) a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ , the  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ , the  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$  and (ii) a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the

chemical shift values of  $^{13}\text{C}'_i$ , the  $\alpha$ -carbon of amino acid residue  $i+1$ ,  $^{13}\text{C}^\alpha_{i+1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i+1$ ,  $^{15}\text{N}_{i+1}$ , and the polypeptide backbone amide proton of amino acid residue  $i+1$ ,  $^1\text{H}^{\text{N}}_{i+1}$ ; and

obtaining sequential assignments by matching the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D HNN<CO,CA> NMR experiment with the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D H,C,C,H-TOCSY,CO,HA NMR experiment.

65. The method according to claim 56, further comprising:

subjecting the protein sample to a RD 3D H,C,C,H-COSY-CO),N,HN NMR experiment to measure and connect the chemical shift values of aliphatic protons of amino acid residue  $i-1$ ,  $^1\text{H}^{\text{ali}}_{i-1}$ , aliphatic carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}^{\text{ali}}_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  for amino acid residues  $i$  having unique pairs of  $^{15}\text{N}_i$  and  $^1\text{H}^{\text{N}}_i$  chemical shift values by matching the chemical shift values of  $^1\text{H}^\alpha$  and  $^{13}\text{C}^\alpha$  measured by said RD 3D HNNCAHA NMR experiment and RD 3D HA,CA,CO),N,HN NMR experiment with the chemical shift values of  $^1\text{H}^\alpha_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D H,C,C,H-COSY-CO),N,HN NMR experiment and using the  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  chemical shift values to identify the type of amino acid residue  $i-1$ .

66. The method according to claim 56 further comprising:

subjecting the protein sample to a RD 3D H,C,C,H-COSY NMR experiment or a RD 3D H,C,C,H-TOCSY NMR experiment to measure and connect the chemical shift values of aliphatic protons of amino acid residue  $i$ ,  $^1\text{H}^{\text{ali}}_i$ , and aliphatic carbons of amino acid residue  $i$ ,  $^{13}\text{C}^{\text{ali}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$  by (i) matching the chemical shift values of  $^1\text{H}^\alpha_i$  and  $^{13}\text{C}^\alpha_i$  measured using said RD 3D H,C,C,H-COSY NMR experiment or RD 3D H,C,C,H-TOCSY RD NMR experiment with the chemical shift values of  $^1\text{H}^\alpha$  and  $^{13}\text{C}^\alpha$  measured by said RD 3D HA,CA,CO),N,HN NMR experiment and RD 3D

HNNCAHA NMR experiment and (ii) using the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$  to identify the type of amino acid residue  $i$ .

67. A method for sequentially assigning chemical shift values of a  $\beta$ -proton,  $^1\text{H}^\beta$ , a  $\beta$ -carbon,  $^{13}\text{C}^\beta$ , an  $\alpha$ -proton,  $^1\text{H}^\alpha$ , an  $\alpha$ -carbon,  $^{13}\text{C}^\alpha$ , a polypeptide backbone amide nitrogen,  $^{15}\text{N}$ , and a polypeptide backbone amide proton,  $^1\text{H}^{\text{N}}$ , of a protein molecule comprising:

providing a protein sample;

conducting a set of reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of  $^1\text{H}^{\alpha/\beta}$  and  $^{13}\text{C}^{\alpha/\beta}$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, comprising: (1) a RD three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}\underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}^\beta_{i-1}$ , the  $\beta$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}^\beta_{i-1}$ , the  $\alpha$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}^\alpha_{i-1}$ , the  $\alpha$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}^\alpha_{i-1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  $^{15}\text{N}_i$ , and the polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}^{\text{N}}_i$  and (2) a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ , the  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ , the  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ ,  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  by (i) matching the chemical shift values of the  $\alpha$ - and  $\beta$ -protons of amino acid residue  $i-1$ ,  $^1\text{H}^{\alpha/\beta}_{i-1}$ , and the  $\alpha$ - and  $\beta$ -carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}^{\alpha/\beta}_{i-1}$ , with the chemical shift values of  $^1\text{H}^{\alpha/\beta}_i$  and  $^{13}\text{C}^{\alpha/\beta}_i$ , (ii) using the chemical shift values of  $^1\text{H}^{\alpha/\beta}_{i-1}$  and  $^{13}\text{C}^{\alpha/\beta}_{i-1}$  to identify the type of amino acid residue  $i-1$ , and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and using said chemical shift values to locate secondary structure elements within the polypeptide chain.

68. The method according to claim 67 further comprising:

subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha}, \underline{C}^{\alpha}, (\text{CO}), \text{N}, \text{HN}$  NMR experiment (i) to measure and connect chemical shift values of  $^1\text{H}^{\alpha}_{i-1}$ ,  $^{13}\text{C}^{\alpha}_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$  and (ii) to distinguish between NMR signals for  $^1\text{H}^{\alpha}/^{13}\text{C}^{\alpha}$  and  $^1\text{H}^{\beta}/^{13}\text{C}^{\beta}$  measured in said RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, (\text{CO})\text{NHN}$  NMR experiment and RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

69. The method according to claim 67 further comprising:

subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ ,  $^1\text{H}^{\alpha}_i$ ,  $^{13}\text{C}^{\alpha}_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_i$  by matching the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ ,  $^1\text{H}^{\alpha}_i$ , and  $^{13}\text{C}^{\alpha}_i$  measured by said RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment with the sequentially assigned chemical shift values of  $^1\text{H}^{\beta}$ ,  $^{13}\text{C}^{\beta}$ ,  $^1\text{H}^{\alpha}$ ,  $^{13}\text{C}^{\alpha}$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  measured by said RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, (\text{CO})\text{NHN}$  NMR experiment and RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

70. The method according to claim 67 further comprising:

subjecting the protein sample to a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the chemical shift values of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ ,  $^{13}\text{C}^{\alpha}_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_{i-1}$  by matching the chemical shift value of  $^{13}\text{C}^{\alpha}_i$  measured by said RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment with the sequentially assigned chemical shift values of  $^{13}\text{C}^{\alpha}$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  measured by said RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, (\text{CO})\text{NHN}$  NMR experiment and RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

71. The method according to claim 67 further comprising:

subjecting the protein sample to (i) a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ ,

$^1\text{H}^\alpha_i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$  and (ii) a RD 3D HNN<CO,CA> NMR experiment to measure and connect the chemical shift values of  $^{13}\text{C}'_i$ , the  $\alpha$ -carbon of amino acid residue  $i+1$ ,  $^{13}\text{C}^\alpha_{i+1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i+1$ ,  $^{15}\text{N}_{i+1}$ , and the polypeptide backbone amide proton of amino acid residue  $i+1$ ,  $^1\text{H}^{\text{N}}_{i+1}$ ; and obtaining sequential assignments by matching the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D HNN<CO,CA> NMR experiment with the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ , $\underline{\text{C}}^{\alpha/\beta}$ ,CO,HA NMR experiment.

72. The method according to claim 67 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}$ , $\underline{\text{C}}$ -(C-TOCSY-CO),N,HN NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$ ,  $^{13}\text{C}^{\text{ali}}_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  for amino acid residues  $i$  having unique pairs of  $^{15}\text{N}_i$  and  $^1\text{H}^{\text{N}}_i$  chemical shift values by matching the chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ , and  $^{13}\text{C}^\alpha$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ , $\underline{\text{C}}^{\alpha/\beta}$ -(CO)NHN NMR experiment and RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ , $\underline{\text{C}}^{\alpha/\beta}$ ,N,HN NMR experiment with the chemical shift values of  $^1\text{H}^\beta_{i-1}$ ,  $^{13}\text{C}^\beta_{i-1}$ ,  $^1\text{H}^\alpha_{i-1}$ , and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D  $\underline{\text{H}}$ , $\underline{\text{C}}$ -(C-TOCSY-CO),N,HN NMR experiment and using the  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  chemical shift values to identify the type of amino acid residue  $i-1$ .

73. The method according to claim 67 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to measure and connect the chemical shift value of  $^{13}\text{C}^\beta_i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and obtaining sequential assignments by matching the chemical shift values of  $^{13}\text{C}^\beta_i$  and  $^{13}\text{C}^\alpha_i$  measured by said 3D HNNCACB NMR experiment with the chemical shift values of  $^{13}\text{C}^\beta_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ , $\underline{\text{C}}^{\alpha/\beta}$ -(CO)NHN NMR experiment.

74. The method according to claim 67 further comprising:  
subjecting the protein sample to a RD two-dimensional (2D) HB, CB, (CG, CD), HD NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ , and a  $\delta$ -proton of amino acid residue  $i$  with an aromatic side chain,  $^1\text{H}^\delta_i$ ; and

obtaining sequential assignments by (i) matching the chemical shift values of  $^1\text{H}^\beta_i$  and  $^{13}\text{C}^\beta_i$  measured by said RD 2D HC, CB, (CG, CD), HDNMR experiment with the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  measured by said RD 3D H $^{\alpha/\beta}$ C $^{\alpha/\beta}$ (CO)NHN NMR experiment and RD 3D H $^{\alpha/\beta}$ , C $^{\alpha/\beta}$ , N, HN NMR experiment, (ii) using said chemical shift values to identify amino acid residue  $i$  as having an aromatic side chain, and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and locating amino acid residues with aromatic side chains along said polypeptide chain.

75. The method according to claim 67, further comprising:  
subjecting the protein sample to a RD 3D H, C, C, H-COSY NMR experiment or a RD 3D H, C, C, H-TOCSY NMR experiment to measure and connect the chemical shift values of aliphatic protons of amino acid residue  $i$ ,  $^1\text{H}^{\text{ali}}_i$  and aliphatic carbons of amino acid residue  $i$ ,  $^{13}\text{C}^{\text{ali}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$  by (i) matching the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ , and  $^{13}\text{C}^\alpha_i$  measured using said RD 3D H, C, C, H-COSY NMR experiment or RD 3D H, C, C, H-TOCSY RD NMR experiment with the chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ , and  $^{13}\text{C}^\alpha$  measured by said RD 3D H $^{\alpha/\beta}$ C $^{\alpha/\beta}$ (CO)NHN NMR experiment and RD 3D H $^{\alpha/\beta}$ , C $^{\alpha/\beta}$ , N, HN NMR experiment, and (ii) using the chemical shift values of  $^1\text{H}^{\text{ali}}_i$  and  $^{13}\text{C}^{\text{ali}}_i$ , to identify the type of amino acid residue  $i$ .

76. A method for sequentially assigning the chemical shift values of aliphatic protons,  $^1\text{H}^{\text{ali}}$ , aliphatic carbons,  $^{13}\text{C}^{\text{ali}}$ , a polypeptide backbone amide nitrogen,  $^{15}\text{N}$ , and a polypeptide backbone amide proton,  $^1\text{H}^{\text{N}}$ , of a protein molecule comprising:

providing a protein sample;

conducting a set of reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of  $^1\text{H}^{\text{ali}}$  and  $^{13}\text{C}^{\text{ali}}$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, comprising: (1) a RD three-dimensional (3D)  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment to measure and connect the chemical shift values of the aliphatic protons of amino acid residue  $i-1$ ,  $^1\text{H}^{\text{ali}}_{i-1}$ , the aliphatic carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}^{\text{ali}}_{i-1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  $^{15}\text{N}_i$ , and the polypeptide backbone amide proton of amino acid residue  $i$ ,  $^1\text{H}^{\text{N}}_i$  and (2) a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment to measure and connect the chemical shift values of the  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^{\beta}_i$ , the  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^{\beta}_i$ , the  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^{\alpha}_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^{\alpha}_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^{\text{ali}}$ ,  $^{13}\text{C}^{\text{ali}}$ ,  $^{15}\text{N}$ , and  $^1\text{H}^{\text{N}}$  by (i) matching the chemical shift values of the  $\alpha$ - and  $\beta$ -protons of amino acid residue  $i-1$ ,  $^1\text{H}^{\alpha/\beta}_{i-1}$  and the  $\alpha$ - and  $\beta$ -carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}^{\alpha/\beta}_{i-1}$  with the chemical shift values of  $^1\text{H}^{\alpha/\beta}_i$  and  $^{13}\text{C}^{\alpha/\beta}_i$  of amino acid residue  $i$ , (ii) using the chemical shift values of  $^1\text{H}^{\text{ali}}_{i-1}$  and  $^{13}\text{C}^{\text{ali}}_{i-1}$  to identify the type of amino acid residue  $i-1$ , and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and using said chemical shift values to locate secondary structure elements within the polypeptide chain.

77. The method according to claim 76 further comprising:

subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ ,  $^1\text{H}^{\alpha}_i$ ,  $^{13}\text{C}^{\alpha}_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_i$  by matching the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ ,  $^1\text{H}^{\alpha}_i$ , and  $^{13}\text{C}^{\alpha}_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment with the sequentially assigned



chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ ,  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^N$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

78. The method according to claim 76 further comprising:  
subjecting the protein sample to a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the chemical shift values of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^N_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_{i-1}$  by matching the chemical shift value of  $^{13}\text{C}^\alpha_i$  measured by said RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment with the sequentially assigned chemical shift values of  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^N$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

79. The method according to claim 76 further comprising:  
subjecting the protein sample to (i) a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$  and (ii) a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the chemical shift values of  $^{13}\text{C}'_i$ , the  $\alpha$ -carbon of amino acid residue  $i+1$ ,  $^{13}\text{C}^\alpha_{i+1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i+1$ ,  $^{15}\text{N}_{i+1}$ , and the polypeptide backbone amide proton of amino acid residue  $i+1$ ,  $^1\text{H}^N_{i+1}$ ; and

obtaining sequential assignments by matching the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment with the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment.

80. The method according to claim 76 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, (\text{CO})\text{NHN}$  NMR experiment (i) to measure and connect the chemical shift values of  $^1\text{H}^{\alpha/\beta}_{i-1}$ ,  $^{13}\text{C}^{\alpha/\beta}_i$ .

$^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ , and (ii) to identify NMR signals for  $^1\text{H}^{\alpha/\beta}_{i-1}$ ,  $^{13}\text{C}^{\alpha/\beta}_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$  in said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment.

81. The method according to claim 76 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \underline{\text{A}}, (\text{CO}), \text{N}, \text{HN}$  NMR experiment (i) to measure and connect chemical shift values of  $^1\text{H}^{\alpha}_{i-1}$ ,  $^{13}\text{C}^{\alpha}_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$  and (ii) to identify NMR signals for  $^1\text{H}^{\alpha}$  and  $^{13}\text{C}^{\alpha}$  in said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment.

82. The method according to claim 76 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to measure and connect the chemical shift value of  $^{13}\text{C}^{\beta}_i$ ,  $^{13}\text{C}^{\alpha}_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^{\text{N}}_i$ ; and  
obtaining sequential assignments by matching the chemical shift values of  $^{13}\text{C}^{\beta}_i$  and  $^{13}\text{C}^{\alpha}_i$  measured by said 3D HNNCACB NMR experiment with the chemical shift values of  $^{13}\text{C}^{\beta}_{i-1}$  and  $^{13}\text{C}^{\alpha}_{i-1}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment.

83. The method according to claim 76 further comprising:  
subjecting the protein sample to a RD two-dimensional (2D)  $\underline{\text{H}}, \underline{\text{B}}, \underline{\text{C}}, \underline{\text{B}}, (\text{CG}, \text{CD}), \text{HD}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^{\beta}_i$ ,  $^{13}\text{C}^{\beta}_i$ , and a  $\delta$ -proton of amino acid residue  $i$  with an aromatic side chain,  $^1\text{H}^{\delta}_i$ ; and  
obtaining sequential assignments by matching the chemical shift values of  $^1\text{H}^{\beta}_i$  and  $^{13}\text{C}^{\beta}_i$  measured by said RD 2D  $\underline{\text{H}}, \underline{\text{B}}, \underline{\text{C}}, \underline{\text{B}}, (\text{CG}, \text{CD}), \text{HD}$  NMR experiment with the chemical shift values of  $^1\text{H}^{\beta}$  and  $^{13}\text{C}^{\beta}$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment and RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment, using said chemical shift values to identify amino acid residue  $i$  as having an aromatic side chain, and mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and

locating amino acid residues with aromatic side chains along said polypeptide chain.

84. The method according to claim 76 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}, \underline{C}, C, H$ -COSY NMR experiment or a RD 3D  $\underline{H}, \underline{C}, C, H$ -TOCSY NMR experiment to measure and connect the chemical shift values of aliphatic protons of amino acid residue  $i$ ,  ${}^1H^{\text{ali}}_i$  and aliphatic carbons of amino acid residue  $i$ ,  ${}^{13}C^{\text{ali}}_i$ ; and  
obtaining sequential assignments of the chemical shift values of  ${}^1H^{\text{ali}}_i$  and  ${}^{13}C^{\text{ali}}_i$  by (i) matching the chemical shift values of  ${}^1H^{\text{ali}}_i$  and  ${}^{13}C^{\text{ali}}_i$  measured using said RD 3D  $\underline{H}, \underline{C}, C, H$ -COSY NMR experiment or RD 3D  $\underline{H}, \underline{C}, C, H$ -TOCSY NMR experiment with the chemical shift values of  ${}^1H^{\text{ali}}$  and  ${}^{13}C^{\text{ali}}$  measured by said RD 3D  $\underline{H}, \underline{C}, (C\text{-TOCSY-CO}), N, HN$  NMR experiment and RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, N, HN$  NMR experiment, and (ii) using the chemical shift values of  ${}^1H^{\text{ali}}_i$  and  ${}^{13}C^{\text{ali}}_i$ , to identify the type of amino acid residue  $i$ .

85. A method for sequentially assigning chemical shift values of aliphatic protons,  ${}^1H^{\text{ali}}$ , aliphatic carbons,  ${}^{13}C^{\text{ali}}$ , a polypeptide backbone amide nitrogen,  ${}^{15}N$ , and a polypeptide backbone amide proton,  ${}^1H^N$ , of a protein molecule comprising:

providing a protein sample;

conducting a set of reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of  ${}^1H^{\text{ali}}$  and  ${}^{13}C^{\text{ali}}$  which are encoded in peak pairs of a 3D NMR spectrum are detected in a phase sensitive manner, comprising: (1) a RD three-dimensional (3D)  $\underline{H}, \underline{C}, (C\text{-TOCSY-CO}), N, HN$  NMR experiment to measure and connect the chemical shift values of the aliphatic protons of amino acid residue  $i-1$ ,  ${}^1H^{\text{ali}}_{i-1}$ , the aliphatic carbons of amino acid residue  $i-1$ ,  ${}^{13}C^{\text{ali}}_{i-1}$ , the polypeptide backbone amide nitrogen of amino acid residue  $i$ ,  ${}^{15}N_i$ , and the polypeptide backbone amide proton of amino acid residue  $i$ ,  ${}^1H^N_i$  and (2) a RD 3D  $HNN\underline{CAHA}$  NMR experiment to measure and connect the chemical shift values of the  $\alpha$ -proton of

amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^\text{N}_i$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}^\text{ali}$ ,  $^{13}\text{C}^\text{ali}$ ,  $^{15}\text{N}$ , and  $^1\text{H}^\text{N}$  by (i) matching the chemical shift values of the  $\alpha$ -proton of amino acid residue  $i-1$ ,  $^1\text{H}^\alpha_{i-1}$ , and the  $\alpha$ -carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}^\alpha_{i-1}$ , with the chemical shift values of  $^1\text{H}^\alpha_i$  and  $^{13}\text{C}^\alpha_i$ , (ii) using the chemical shift values of  $^1\text{H}^\text{ali}_{i-1}$  and  $^{13}\text{C}^\text{ali}_{i-1}$  to identify the type of amino acid residue  $i-1$ , and (iii) mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and using said chemical shift values to locate secondary structure elements within the polypeptide chain.

86. The method according to claim 85 further comprising:

subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ ,  $\underline{\text{C}}^{\alpha/\beta}$ , CO, HA NMR experiment to measure and connect the chemical shift values of a  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ , a  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_i$  by matching the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ , and  $^{13}\text{C}^\alpha_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ ,  $\underline{\text{C}}^{\alpha/\beta}$ , CO, HA NMR experiment with the sequentially assigned chemical shift values of  $^1\text{H}^\beta$ ,  $^{13}\text{C}^\beta$ ,  $^1\text{H}^\alpha$ ,  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^\text{N}$  measured by said RD 3D  $\underline{\text{H}}$ ,  $\underline{\text{C}}$ , (C-TOCSY-CO), N, HN NMR experiment and RD 3D HNN CAHA NMR experiment.

87. The method according to claim 85 further comprising:

subjecting the protein sample to a RD 3D HNN<CO, CA> NMR experiment to measure and connect the chemical shift values of a polypeptide backbone carbonyl carbon of amino acid residue  $i-1$ ,  $^{13}\text{C}'_{i-1}$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^\text{N}_i$ ; and

obtaining sequential assignments of the chemical shift value of  $^{13}\text{C}'_{i-1}$  by matching the chemical shift value of  $^{13}\text{C}^\alpha_i$  measured by said RD 3D HNN<CO, CA> NMR experiment with the sequentially assigned chemical shift

values of  $^{13}\text{C}^\alpha$ ,  $^{15}\text{N}$ , and  $^1\text{H}^\text{N}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\text{HNNCAHA}$  NMR experiment.

88. The method according to claim 85 further comprising:  
subjecting the protein sample to (i) a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment to measure and connect the chemical shift values of a  $\beta$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\beta_i$ , a  $\beta$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\beta_i$ , the  $\alpha$ -proton of amino acid residue  $i$ ,  $^1\text{H}^\alpha_i$ , the  $\alpha$ -carbon of amino acid residue  $i$ ,  $^{13}\text{C}^\alpha_i$ , and a polypeptide backbone carbonyl carbon of amino acid residue  $i$ ,  $^{13}\text{C}'_i$  and (ii) a RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment to measure and connect the chemical shift values of  $^{13}\text{C}'_i$ , an  $\alpha$ -carbon of amino acid residue  $i+1$ ,  $^{13}\text{C}^\alpha_{i+1}$ , a polypeptide backbone amide nitrogen of amino acid residue  $i+1$ ,  $^{15}\text{N}_{i+1}$ , and a polypeptide backbone amide proton of amino acid residue  $i+1$ ,  $^1\text{H}^\text{N}_{i+1}$ ; and  
obtaining sequential assignments by matching the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D  $\text{HNN} < \underline{\text{CO}}, \underline{\text{CA}} >$  NMR experiment with the chemical shift value of  $^{13}\text{C}'_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{CO}, \text{HA}$  NMR experiment.

89. The method according to claim 85 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment (i) to measure and connect the chemical shift values of the  $\alpha$ - and  $\beta$ -protons of amino acid residue  $i-1$ ,  $^1\text{H}^{\alpha/\beta}_{i-1}$ ,  $\alpha$ - and  $\beta$ -carbons of amino acid residue  $i-1$ ,  $^{13}\text{C}^{\alpha/\beta}_{i-1}$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^\text{N}_i$ , and (ii) to distinguish NMR signals for the chemical shift values of  $^1\text{H}^\beta_{i-1}$ ,  $^{13}\text{C}^\beta_{i-1}$ ,  $^1\text{H}^\alpha_{i-1}$ , and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment from NMR signals for the chemical shift values of  $^1\text{H}^\text{ali}_{i-1}$  and  $^{13}\text{C}^\text{ali}_{i-1}$  other than  $^1\text{H}^{\alpha/\beta}_{i-1}$  and  $^{13}\text{C}^{\alpha/\beta}_{i-1}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment.

90. The method according to claim 85 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^\text{N}_i$ ; and

obtaining sequential assignments by matching the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ ,  $^1\text{H}^\alpha_i$ , and  $^{13}\text{C}^\alpha_i$  measured by said RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment with the chemical shift values of  $^1\text{H}^\beta_{i-1}$ ,  $^{13}\text{C}^\beta_{i-1}$ ,  $^1\text{H}^\alpha_{i-1}$ , and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment.

91. The method according to claim 85 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to measure and connect the chemical shift values of  $^{13}\text{C}^\beta_i$ ,  $^{13}\text{C}^\alpha_i$ ,  $^{15}\text{N}_i$ , and  $^1\text{H}^\text{N}_i$ ; and

obtaining sequential assignments by matching the chemical shift values of  $^{13}\text{C}^\beta_i$  and  $^{13}\text{C}^\alpha_i$  measured by said 3D HNNCACB NMR experiment with the chemical shift values of  $^{13}\text{C}^\beta_{i-1}$  and  $^{13}\text{C}^\alpha_{i-1}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment.

92. The method according to claim 85 further comprising:  
subjecting the protein sample to a RD two-dimensional (2D)  $\underline{\text{HB}}, \underline{\text{CB}}, (\text{CG}, \text{CD}), \text{HD}$  NMR experiment to measure and connect the chemical shift values of  $^1\text{H}^\beta_i$ ,  $^{13}\text{C}^\beta_i$ , and a  $\delta$ -proton of amino acid residue  $i$  with an aromatic side chain,  $^1\text{H}^\delta_i$ ; and

obtaining sequential assignments by matching the chemical shift values of  $^1\text{H}^\beta_i$  and  $^{13}\text{C}^\beta_i$  measured by said RD 2D  $\underline{\text{HB}}, \underline{\text{CB}}, (\text{CG}, \text{CD}), \text{HD}$  NMR experiment with the chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment, using said chemical shift values to identify amino acid residue  $i$  as having an aromatic side chain, and mapping sets of sequentially connected chemical shift values to the amino acid sequence of the polypeptide chain and locating amino acid residues with aromatic side chains along said polypeptide chain.

93. The method according to claim 85 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H-COSY}$  NMR experiment or a RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H-TOCSY}$  NMR experiment to measure and

connect the chemical shift values of aliphatic protons of amino acid residue  $i$ ,  $^1\text{H}_i^{\text{ali}}$  and aliphatic carbons of amino acid residue  $i$ ,  $^{13}\text{C}_i^{\text{ali}}$ ; and

obtaining sequential assignments of the chemical shift values of  $^1\text{H}_i^{\text{ali}}$  and  $^{13}\text{C}_i^{\text{ali}}$  by (i) matching the chemical shift values of  $^1\text{H}_i^{\text{ali}}$  and  $^{13}\text{C}_i^{\text{ali}}$  measured using said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H}$ -COSY NMR experiment or RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H}$ -TOCSY NMR experiment with the chemical shift values of  $^1\text{H}_i^{\beta}$ ,  $^{13}\text{C}_i^{\beta}$ ,  $^1\text{H}_i^{\alpha}$ , and  $^{13}\text{C}_i^{\alpha}$  measured by said RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment and RD 3D  $\text{HNNCAHA}$  NMR experiment, and (ii) using the chemical shift values of  $^1\text{H}_i^{\text{ali}}$  and  $^{13}\text{C}_i^{\text{ali}}$  to identify the type of amino acid residue  $i$ .

94. A method for obtaining assignments of chemical shift values of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  of a protein molecule comprising:

providing a protein sample; and

conducting four reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  which are encoded in peak pairs of an NMR spectrum are detected in a phase sensitive manner and (1) a first experiment is selected from the group consisting of a RD three-dimensional (3D)  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment, a RD 3D  $\underline{\text{HA}}, \underline{\text{CA}}, (\text{CO}), \text{N}, \text{HN}$  NMR experiment, and a RD 3D  $\underline{\text{H}}, \underline{\text{C}}, (\text{C-TOCSY-CO}), \text{N}, \text{HN}$  NMR experiment for obtaining sequential correlations of chemical shift values; (2) a second experiment is selected from the group consisting of a RD 3D  $\text{HNNCAHA}$  NMR experiment, a RD 3D  $\underline{\text{H}}^{\alpha/\beta}, \underline{\text{C}}^{\alpha/\beta}, \text{N}, \text{HN}$  NMR experiment, and a RD 3D  $\text{HNN} \langle \underline{\text{CO}}, \underline{\text{CA}} \rangle$  NMR experiment for obtaining intraresidue correlations of chemical shift values; (3) a third experiment is a RD 3D  $\underline{\text{H}}, \underline{\text{C}}, \text{C}, \text{H}$ -COSY NMR experiment for obtaining assignments of sidechain chemical shift values; and (4) a fourth experiment is a RD two-dimensional (2D)  $\underline{\text{HB}}, \underline{\text{CB}}, (\text{CG}, \text{CD}), \text{HD}$  NMR experiment for obtaining assignments of aromatic sidechain chemical shift values.

95. The method according to claim 94 further comprising:

subjecting the protein sample to a RD 2D  $\underline{\text{H}}, \underline{\text{C}}, \text{H}$ -COSY NMR experiment for obtaining assignments of sidechain chemical shift values.

96. The method according to claim 94, wherein the first experiment is the RD 3D  $\underline{H}^{\alpha/\beta}\underline{C}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment and the second experiment is the RD 3D  $\text{HNNCAHA}$  NMR experiment.

97. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{HA},\underline{CA},(\text{CO}),\text{N},\text{HN}$  NMR experiment to distinguish between NMR signals for  $^1\text{H}^\alpha/^{13}\text{C}^\alpha$  and  $^1\text{H}^\beta/^{13}\text{C}^\beta$  from said RD 3D  $\underline{H}^{\alpha/\beta}\underline{C}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR experiment.

98. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H},\underline{C},(\text{C-TOCSY-}\text{CO}),\text{N},\text{HN}$  NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}^{\text{ali}}$  and  $^{13}\text{C}^{\text{ali}}$ .

99. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta},\underline{C}^{\alpha/\beta},\text{N},\text{HN}$  NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$ .

100. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\text{HNN}<\underline{\text{CO}},\underline{\text{CA}}>$  NMR experiment to obtain assignments of chemical shift values of polypeptide backbone carbonyl carbons,  $^{13}\text{C}'$ .

101. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta},\underline{C}^{\alpha/\beta},\text{CO},\text{HA}$  NMR experiment to obtain assignments of chemical shift values of polypeptide backbone carbonyl carbons,  $^{13}\text{C}'$ .

102. The method according to claim 96 further comprising:



subjecting the protein sample to a RD 3D HNN<CO,CA> NMR experiment and a RD 3D  $\underline{H}^{\alpha/\beta}$ , $\underline{C}^{\alpha/\beta}$ ,CO,HA NMR experiment to obtain assignments of chemical shift values of  $^{13}\text{C}'$ .

103. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}$ , $\underline{C}$ ,C,H-TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of aliphatic sidechains.

104. The method according to claim 96 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}$ , $\underline{C}$ ,C,H-TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of aromatic sidechains.

105. The method according to claim 96 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to obtain assignments of chemical shift values of  $^{13}\text{C}^\beta$ .

106. The method according to claim 96, wherein the first experiment is the RD 3D  $\underline{H}$ , $\underline{C}$ , (C-TOCSY-CO),N,HN NMR experiment and the second experiment is the RD 3D HNNCAHA NMR experiment.

107. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D HA,CA, (CO),N,HN NMR experiment to identify NMR signals for  $^1\text{H}^\alpha/^{13}\text{C}^\alpha$  in said RD 3D  $\underline{H}$ , $\underline{C}$ , (C-TOCSY-CO),N,HN NMR experiment.

108. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta}$ , $\underline{C}^{\alpha/\beta}$ ,N,HN NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}^\beta$  and  $^{13}\text{C}^\beta$ .

109. The method according to claim 106 further comprising:

subjecting the protein sample to a RD 3D HNN<CO,CA> NMR experiment to obtain assignments of chemical shift values of polypeptide backbone carbonyl carbons,  $^{13}\text{C}'$ .

110. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ ,  $\underline{\text{C}}^{\alpha/\beta}$ , CO, HA NMR experiment to obtain assignments of chemical shift values of polypeptide backbone carbonyl carbons,  $^{13}\text{C}'$ .

111. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D HNN<CO,CA> NMR experiment and a RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ ,  $\underline{\text{C}}^{\alpha/\beta}$ , CO, HA NMR experiment to obtain assignments of chemical shift values of  $^{13}\text{C}'$ .

112. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}$ ,  $\underline{\text{C}}$ , C, H-TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of aliphatic sidechains.

113. The method according to claim 106 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}$ ,  $\underline{\text{C}}$ , C, H-TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of aromatic sidechains.

114. The method according to claim 106 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to obtain assignments of chemical shift values of  $^{13}\text{C}^{\beta}$ .

115. The method according to claim 94, wherein the first experiment is the RD 3D  $\underline{\text{H}}$ ,  $\underline{\text{C}}$ , (C-TOCSY-CO), N, HN NMR experiment and the second experiment is the RD 3D  $\underline{\text{H}}^{\alpha/\beta}$ ,  $\underline{\text{C}}^{\alpha/\beta}$ , N, HN NMR experiment.

116. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}\underline{\text{A}},\underline{\text{C}}\underline{\text{A}},(\text{CO}),\text{N},\text{HN}$   
NMR experiment to identify NMR signals for  $^1\text{H}^\alpha$  and  $^{13}\text{C}^\alpha$  in said RD 3D  
 $\underline{\text{H}},\underline{\text{C}},(\text{C-TOCSY-CO}),\text{N},\text{HN}$  NMR experiment.

117. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta},\underline{\text{C}}^{\alpha/\beta}(\text{CO})\text{NHN}$  NMR  
experiment to identify NMR signals for  $^1\text{H}^{\alpha/\beta}$  and  $^{13}\text{C}^{\alpha/\beta}$  in said RD 3D  $\underline{\text{H}},\underline{\text{C}},(\text{C-TOCSY-CO}),\text{N},\text{HN}$  NMR experiment.

118. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\text{HNN}<\underline{\text{CO}},\underline{\text{CA}}>$  NMR  
experiment to obtain assignments of chemical shift values of polypeptide  
backbone carbonyl carbons,  $^{13}\text{C}'$ .

119. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}}^{\alpha/\beta},\underline{\text{C}}^{\alpha/\beta},\text{CO},\text{HA}$  NMR  
experiment to obtain assignments of chemical shift values of polypeptide  
backbone carbonyl carbons,  $^{13}\text{C}'$ .

120. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\text{HNN}<\underline{\text{CO}},\underline{\text{CA}}>$  NMR  
experiment and a RD 3D  $\underline{\text{H}}^{\alpha/\beta},\underline{\text{C}}^{\alpha/\beta},\text{CO},\text{HA}$  NMR experiment to obtain  
assignments of chemical shift values of  $^{13}\text{C}'$ .

121. The method according to claim 115 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{\text{H}},\underline{\text{C}},\text{C},\text{H-TOCSY}$  NMR  
experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of  
aliphatic sidechains.

122. The method according to claim 115 further comprising:

subjecting the protein sample to a RD 3D  $\underline{H}, \underline{C}, C, H$ -TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1H$  and  $^{13}C$  of aromatic sidechains.

123. The method according to claim 115 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR experiment to obtain assignments of chemical shift values of  $^{13}C^\beta$ .

124. The method according to claim 94, wherein the first experiment is the RD 3D  $\underline{H}, \underline{C}, (C\text{-TOCSY-CO}), N, HN$  NMR experiment and the second experiment is the RD 3D HNN< $\underline{CO}, \underline{CA}$ > NMR experiment.

125. The method according to claim 124 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{HA}, \underline{CA}, (CO), N, HN$  NMR experiment to identify NMR signals for  $^1H^\alpha$  and  $^{13}C^\alpha$  in said RD 3D  $\underline{H}, \underline{C}, (C\text{-TOCSY-CO}), N, HN$  NMR experiment.

126. The method according to claim 124 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta} (CO) NHN$  NMR experiment to identify NMR signals for  $^1H^{\alpha/\beta}$  and  $^{13}C^{\alpha/\beta}$  in said RD 3D  $\underline{H}, \underline{C}, (C\text{-TOCSY-CO}), N, HN$  NMR experiment.

127. The method according to claim 124 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}^{\alpha/\beta}, \underline{C}^{\alpha/\beta}, CO, HA$  NMR experiment to obtain assignments of chemical shift values of polypeptide backbone carbonyl carbons,  $^{13}C'$ .

128. The method according to claim 124 further comprising:  
subjecting the protein sample to a RD 3D  $\underline{H}, \underline{C}, C, H$ -TOCSY NMR experiment to obtain assignments of chemical shift values of  $^1H$  and  $^{13}C$  of aliphatic sidechains.

129. The method according to claim 124 further comprising:  
subjecting the protein sample to a RD 3D  $\text{H}_\alpha\text{C}_\alpha\text{H}$ -TOCSY NMR  
experiment to obtain assignments of chemical shift values of  $^1\text{H}$  and  $^{13}\text{C}$  of  
aromatic sidechains.

130. The method according to claim 124 further comprising:  
subjecting the protein sample to a 3D HNNCACB NMR  
experiment to obtain assignments of chemical shift values of  $^{13}\text{C}^\beta$ .

131. The method according to claim 94 further comprising:  
subjecting the protein sample to nuclear Overhauser effect  
spectroscopy (NOESY) to deduce the tertiary structure of the protein molecule.

132. The method according to claim 94 further comprising:  
subjecting the protein sample to NMR experiments that measure  
scalar coupling constants to deduce the tertiary structure of the protein molecule.

133. The method according to claim 94 further comprising:  
subjecting the protein sample to NMR experiments that measure  
residual dipolar coupling constants to deduce the tertiary structure of the protein  
molecule.